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Ratio of Serum γ-GT/ALT Rather Than ISDR Variability Is Predictive for Initial Virological Response to IFN-α in Chronic HCV Infection

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Chronic hepatitis C virus (HCV) infection in humans is treated at present with interferon (IFN)-α. Because the proportion of patients responding to therapy with sustained or even just with transient elimination of viral RNA is low, several potential prognostic parameters have been evaluated to predict the outcome of the therapy. The present study aimed to prove the validity of a predictive parameter described previously for initial virological response, namely the ratio of serum γ-glutamyltransferase/alanine transaminase (γ-GT/ALT) activity in connection with virus genotypes 1a, 1b, and 3a, prospectively and to compare the predictive value of these combined parameters with amino acid variability within the interferon sensitivity determining region (ISDR). The prospective analysis confirmed previous data on the predictive value of the serum γ-GT/ALT ratio. Concerning ISDR variability, the majority of ISDR sequences obtained from the mostly nonresponding type 1b-infected individuals (23/28) resembled nonmutant types (27/28). Isolates from type 3a-infected patients responding to therapy in the majority of cases (13/20) exclusively resembled nonmutant types when compared with databank type 3a sequences, but were mutant when compared with the prototype sequence HCV-J. However, the initial virological responsiveness among both type 1b- and type 3a-infected patients did not correlate to ISDR variability. In contrast, virological responsiveness was closely related to serum γ-GT/ALT ratio. The data are not necessarily contrary to the concept that the number of amino acid exchanges within the ISDR compared with the prototype HCV-J sequence is related to some extent to IFN-α sensitivity. The ratio of serum γ-GT/ALT in combination with HCV genotype, however, was found to be a more reliable and stringent predictive parameter. ***J. Med. Virol. 58: 227–234, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: HCV; IFN-α; γ-GT/ALT; interferon sensitivity determining region

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded plus-sense RNA virus that causes acute and often also chronic liver disease [Choo et al., 1989; Kuo et al., 1989; Kolykhalov et al., 1997]. Its prevalence has been estimated to range between 0.3% and 4.0% worldwide [Sherlock, 1992; Nishioka, 1994]. After a latency of 20–30 years, persons infected chronically with HCV may develop liver cirrhosis and/or hepatocellular carcinoma [Saito et al., 1990; Shimotohno, 1993].

At present, interferon-α (IFN-α) is the treatment of choice. IFN-α has been shown to exert antiinflammatory and antiviral effects in patients infected chronically with hepatitis B virus (HBV) [Greenberg et al., 1976; Wong et al., 1993] and in patients with chronic non-A, non-B hepatitis [Hoofnagle et al., 1986]. A meta-analysis of 27 randomized, controlled trials also proved its efficacy for chronic HCV infection [Malaguarnera et al., 1995]. However, although most of the patients benefit in terms of at least transient normalization of biochemical liver function tests, only minor benefits have been observed in terms of a transient or a sustained elimination of viral nucleic acids from the circulation [Tine et al., 1991; Saracco and Rizetto, 1995; Cammà et al., 1997].

Several attempts have been made to identify prognostic markers for successful outcome of therapy, including both pretreatment host and virus characteristics [reviewed by Davis, 1994]. Despite difficulties of comparing single studies due to geographical differ-

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ences in genotype distribution, the virus genotype seemed to be the most reliable [Davis, 1994; Mihm et al., 1996]. Within the group of type 1b-infected patients, however, viral load seems to be an additional independent [Chayama et al., 1997; Sáiz et al., 1998] although weak predictor [Pawlotsky et al., 1998].

We have studied patients infected with HCV types 1a, 1b, and 3a. The importance of the virus genotype for responsiveness to IFN-α therapy was confirmed. In addition, another host-specific parameter with a potential predictive value was found, namely the pretreatment ratio of serum γ-GT/ALT [Mihm et al., 1996]. This ratio was shown to allow the selection of virologically nonresponding patients from those who eliminate the virus at least transiently from circulation, e.g., from potentially long-term responding patients, with a positive predictive value of $P_{pos} = 1$ and a negative predictive value of $P_{neg} = 0.833$ [Mihm et al., 1996]. Among the patients under investigation, the virus titer did not correlate with an early response, nor with the genotype or to the γ-GT/ALT ratio [Mihm et al., 1996].

Analyzing the virological response in a well-defined group of patients infected exclusively with HCV genotype 1b, Enomoto et al. [1995] identified a so-called interferon sensitivity determining region (ISDR) within the coding region of the nonstructural protein NS5A by a direct sequencing procedure. A high variability in this region was found to be associated with increased sensitivity to IFN-α, whereas viruses that lack mutations compared with the prototype isolate HCV-J were found to be more resistant [Enomoto et al., 1995, 1996]. Independently and applying the same technique, two other groups obtained comparable results [Chayama et al., 1997; Sáiz et al., 1998]. Enomoto et al. [1996] were able to extend their findings and found that patients infected with HCV type 2a and 2b, respectively, showed a rate of complete response comparable to patients infected with mutant type 1b isolates [Kurosaki et al., 1997]. However, three European groups were not able to confirm the predictive value of the variability within the ISDR region for patients infected with HCV genotypes 1b or 1a, respectively [Khorsi et al., 1997; Squadrito et al., 1997; Zeuzem et al., 1997].

The aim of the present study was to ascertain the prognostic value of serum γ-GT/ALT ratio for IFN-α responsiveness in a prospective study. Moreover, the ratio of serum γ-GT/ALT activity was opposed to variability within the ISDR region with respect to the initial virological response. Sequence analyses of the region spanning the putative ISDR in HCV genotype 3a isolates were also included.

MATERIAL AND METHODS

Patients

A total of 65 patients infected chronically with HCV as diagnosed by the presence of anti-HCV antibodies and HCV RNA in serum were studied consecutively (24 women, 41 men; aged 23–69 years, mean 44.8 years). Chronicity was confirmed histopathologically according to established criteria [Mihm et al., 1997]. In patients who refused liver biopsy, chronicity was documented by longitudinal observation; the presence of severe liver disease was judged unlikely on the basis of noninvasive studies. Patients with concomitant active HBV or HIV infection and those with continued alcohol or drug abuse were excluded. After informed consent was obtained, treatment with recombinant human IFN-α (Roferon A, Hoffmann-La Roche, Basel, Switzerland) was initiated at a dose of 3×10^6 or 6×10^6 IU thrice weekly and continued for at least 5 months. Depending on well being and response parameters, both dose and duration were adapted individually. The study was approved by the local ethical committee of the Georg-August-University, Göttingen, Germany.

Forty-eight patients infected with genotypes 1a, 1b, or 3a were included in the prospective evaluation of virological response (19 women, 29 men; aged 23–69 years, mean 43.7 years). Before treatment, a prognosis regarding the initial virological response on the basis of HCV genotype and the ratio of γ-GT/ALT was noted as described elsewhere [Mihm et al., 1996]. Therapy was undertaken without the treating doctor's knowledge of the documented prognosis. The outcome of therapy was recorded and analyzed after treatment with IFN-α was stopped.

Twenty-eight patients infected with HCV genotype 1b and 20 patients infected with HCV genotype 3a were included in the comparison of ISDR variability and serum γ-GT/ALT ratio for initial response. Clinical data from these patients are summarized in Table I.

Initial virological response was defined as the disappearance of viral nucleic acid from circulation below the limit of detection for at least 3 consecutive months during treatment. The presence of serum HCV-RNA was assayed in duplicate using a nested reverse transcription-polymerase chain reaction (RT-PCR) procedure [Mihm et al., 1996]. Enzymatic activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (γ-GT) were analyzed by automated routine systems by the central laboratory of the Department of Clinical Chemistry.

Determination of HCV Genotypes

Determination of HCV genotypes was carried out according to Okamoto et al. [1992, 1993] with a subsequent restriction enzyme analysis as described previously [Mihm et al., 1996].

Determination of Nucleotide and Deduced Amino Acid Sequences Within $NS5A_{2209–2248/2215–2254}$

RNA was isolated from 200 μl serum using a commercially available extraction kit (RNA-Clean system, AGS, Heidelberg, Germany). One-fifth of the RNA preparation was subjected to reverse transcription. The reaction was performed at 42°C for 30 min in a total volume of 20 μl containing 10 mM Hepes pH 6.9, 0.2 mM ethylenediamine tetraacetic acid (EDTA) pH 8,

TABLE I. Summarized Clinical Data From Patients Enrolled Into the Comparison of ISDR Variability and the Serum γ-GT/ALT Ratio Regarding IFN-α Responsiveness

	Genotype 1b			Genotype 3a		
	VR	NVR	*P*	VR	NVR	*P*
n	5	23		13	7	
Ratio γ-GT/ALT	0.31	1.00	.0015	0.26	0.60	.0013
Serum γ-GT [U/l]	14	43	.1328	21	30	.1817
Serum ALT [U/l]	45	34	.5885	84	50	.0259
Age (years)	39	54	.1515	35	34	.7880
Dose IFN-α ($\times 10^6$ IU)	333	368	.6240	208	388	.0016

ISDR, interferon sensitivity-determining region; GT, glutamyltransferase; ALT, alanine transaminase; IFN, interferon; VR, viral therapy responders; NVR, nonresponders to viral therapy.
The given data correspond to patients whose ISDR variability is documented in Figures 2 and 3. Medians of the serum γ-GT/ALT ratio, serum γ-GT, serum ALT, and age are shown. The given dose of IFN-α resembles the mean value of the amount of the total dose administered until the end of treatment or until transient virus elimination had been achieved. The lower total dose of IFN-α having been administered to responding type 3a-infected patients thus just reflects a more early response to the drug compared with responding type 1b-infected patients. An univariate logistic regression analysis revealed that the impact of serum γ-GT/ALT ratio was the most significant for drug responsiveness.

50 mM Tris-Cl pH 7.5, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 0.5 mM dNTP, 20 U RNase inhibitor (RNA-guard, Pharmacia, Freiburg, Germany), 15 U Superscript II-RT (GIBCO/BRL, Eggenstein, Germany), and 50 ng of the genotype 1b-specific primer Geno1b/2 (5′-TCTTT- CTCCGTGGAGGTGG-TATTGG-3′) or of the type 3a-specific primer Geno3/3 (5′-GTCCG-GTCTAGCCCAGATAG-3′), respectively. The reaction was stopped by heat inactivation. One-fourth of the cDNA was amplified by PCR carrying out 30 cycles with denaturation for 30 sec at 94°C, primer annealing for 40 sec at 60°C, and extension for 60 sec at 72°C. Amplification was followed by a final extension step for 7 min at 72°C. Each reaction contained 10 mM Tris-Cl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dNTP, 0.5 U Taq-poly-merase (Boehringer Mannheim, Mannheim, Germany), and 30 ng of each primer of the primer pairs Geno1b/1 (5′-TGGATGG-AGTGCGGTTGCACAGGTA-3′ sense) and Geno1b/2, and Geno3/1 (5′-CHGTGCTGACCTCGATGTTG-3′ sense) and Geno3/3 for type 1b or 3a specific amplification, respectively, in a total volume of 50 μl. Starting with 1 μl of the first-round product, a second amplification reaction was performed using 125 ng of the internal primer pairs Geno3/2 (5′-GCGCGCGGGTC-CCCTCCATC-3′ sense) and Geno3/3, and Geno1b/3 (5′-CAGGTACGCTCCGGCGTGCA-3′ sense) and Geno1b/4 (5′-GGGGCCTT-GGTAGGTGGCAA-3′ antisense), respectively. One-tenth of the second-round product was analyzed by agarose gel electrophoresis.

Amplification products were purified by removing excess nucleotides and oligonucleotides using a commercially available purification kit (QIAquick PCR purification kit, QIAGEN, Hilden, Germany). Nucleotide sequences were determined for both strands with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer, Weiterstadt, Germany) on an ABI 373A DNA sequencer (Applied Biosystems, Weiterstadt, Germany). Sequences were analyzed using the program package GCG (Wisconsin Sequence Analysis Package, Version 8.1, Wisconsin).

Statistical Analyses

Univariate and stepwise logistic regression analyses (step-up procedure) were undertaken to detect the predictive meaning of independent variables for initial virological responsiveness to an IFN-α therapy. Fisher's exact test was applied to determine the level of significance of the calculated positive and negative predictive values in the prospective trial.

RESULTS

The Ratio of Serum γ-GT/ALT Activity in Combination with HCV Genotype Is Indicative for Early Virological Responsiveness to IFN-α Therapy

Previous results demonstrating a preferential virological response to IFN-α depending on the HCV genotype and the ratio of serum γ-GT/ALT in retrospect suggested that patients infected with genotypes 1a, 1b, and 3a and pretreatment serum γ-GT/ALT ratios below 0.3, 0.4, and 0.5, respectively, should have a good prognosis for an initial virological response to IFN-α [Mihm et al., 1996]. Applying these criteria, the predictive value of this combined potential prognostic parameter was confirmed prospectively. Forty-eight patients with chronic HCV infection of genotype 1a, 1b, and 3a were included, 17 of whom met the criteria for a positive prognosis and 31 of whom expected not to respond to therapy with transient virus elimination (Fig. 1). Each of the 17 patients with a favorable prognosis did respond to therapy with the disappearance of viral nucleic acids from the circulation for at least 3 consecutive months, yielding a positive predictive value (P_{pos}) of 1. Among the remaining 31 patients with a negative prognosis regarding initial virological response, 25 actually failed to respond, yielding a negative predictive value (P_{neg}) of 0.81 ($P < .0001$, Fisher's exact test).

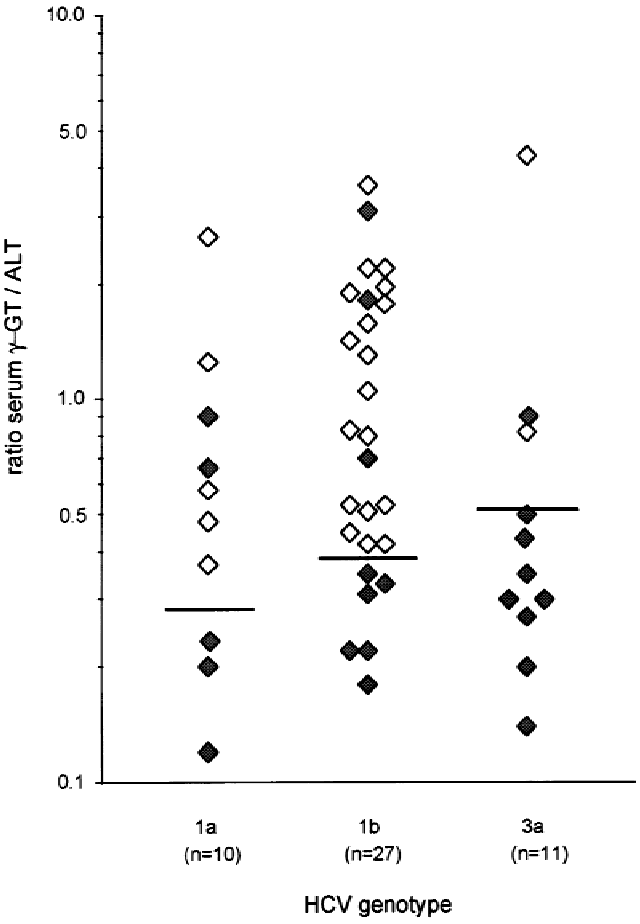

Fig. 1. Prospective analysis of initial virological response to interferon (IFN)-α therapy in relation to virus genotype and serum γ-glutamyltransferase/alanine transaminase (γ-GT/ALT) ratio. According to the previously suggested criteria for initial virological responsiveness, patients infected by hepatitis C virus (HCV) genotype 1a, 1b, and 3a and a serum γ-GT/ALT ratio below 0.3, 0.4, and 0.5 as indicated were predicted to respond, whereas patients with serum γ-GT/ALT ratios above 0.3, 0.4, and 0.5, respectively, should fail to respond. Actual initial virological response (♦) and nonresponse to therapy (◇) are indicated. Each patient with a positive prognosis was found to respond to therapy. Patients with a negative prognosis failed to respond to therapy, except for 6 of them. Statistical analysis of the probability of response and nonresponse thus revealed a positive predictive value (P_{pos}) of 1 and a negative predictive value (P_{neg}) of 0.81.

The Initial Virological Response to IFN-α in Genotype 1b- and in Genotype 3a-Infected Patients Is More Closely Related to Serum γ-GT/ALT Ratio than to ISDR Variability

ISDR variability was determined in 28 patients infected with HCV type 1b and in 20 patients infected with type 3a by analyzing nucleotide sequence and comparing the deduced amino acid sequence with consensus sequences from type 1b and 3a prototypes, respectively. Within the group of type 1b-infected patients, 5/28 (17.9%) were found to respond to IFN-α therapy with virus elimination from the serum below the limit of detection for at least 3 consecutive months (Fig. 2). Sequence analysis revealed prototype sequences in 4 of the nonresponding and in 1 of the virologically responding patients, and intermediate types as so designated by several researchers [Enomoto et al., 1995, 1996; Chayama et al., 1997; Pawlotsky et al., 1998] with 1–3 amino acid substitutions in 19 nonresponding and 3 of the responding patients (Fig. 2). The only mutant type sequence with 4 or more substitutions was detected among patients who responded virologically (Fig. 2). Analysis of the ratio of γ-GT/ALT revealed that all with the exception of 2 of the nonresponding patients had a ratio above 0.4 and that all with the exception of 1 of the responding patients showed a ratio below 0.4 (Fig. 2).

Within the group of patients infected with HCV type 3a, 13/20 (65%) responded to therapy with an at least transient elimination of viral nucleic acids from serum (Fig. 3). Compared with HCV type 3a database sequences, a maximum of 2 amino acid substitutions was detected (Fig. 3). No difference in the absolute number of amino acid exchanges could be observed between responding and nonresponding individuals (Fig. 3). Again, with the exception of 1 patient each, a serum γ-GT/ALT ratio below 0.5 was found to be associated with initial virological response, whereas patients with a ratio above 0.5 did not respond to therapy with transient virus elimination (Fig. 3). Compared with HCV type 1b prototype sequence, which differs from type 3a amino acid sequences in 8 positions, however, the number of amino acid exchanges ranges from 8 up to 10 (Fig. 3).

Stepwise logistic regression analyses (step-up procedure) were carried out to prove the superiority of serum γ-GT/ALT ratio as a predictive parameter compared with other parameters known to be important for IFN-α responsiveness. For instance, the predictive meaning of serum ratio of γ-GT/ALT activity ($P = .0015$ for type 1b-infected patients, $P = .0013$ for type 3a-infected patients) (Table I) could not be further improved by including ISDR variability ($P = .8800$ for type 1b patients, $P = .4502$ for type 3a patients), age ($P = .3756$ for type 1b patients, $P = .8347$ for 3a patients), and the degree of fibrosis ($P = .9781$ for type 1b patients, $P = .5840$ for type 3a patients). Applying other logistic regression models, e.g., including absolute serum γ-GT and ALT activities, or histological parameters such as fibrosis, steatosis, and activity of hepatitis, comparable nonsignificant results were obtained (data not shown).

DISCUSSION

The data described above confirmed by a prospective study a potential prognostic parameter for virological responsiveness to IFN-α therapy in HCV-infected patients, namely the ratio of serum γ-GT/ALT in combination with HCV genotype [Mihm et al., 1996]. This prognostic parameter has been shown to be valid for patients infected with HCV types 1a, 1b, and 3a independently from pretreatment viral load [Mihm et al., 1996]. More importantly, pretreatment ratio of serum γ-GT/ALT activity was shown to be more closely related to virological responsiveness than ISDR variabil-

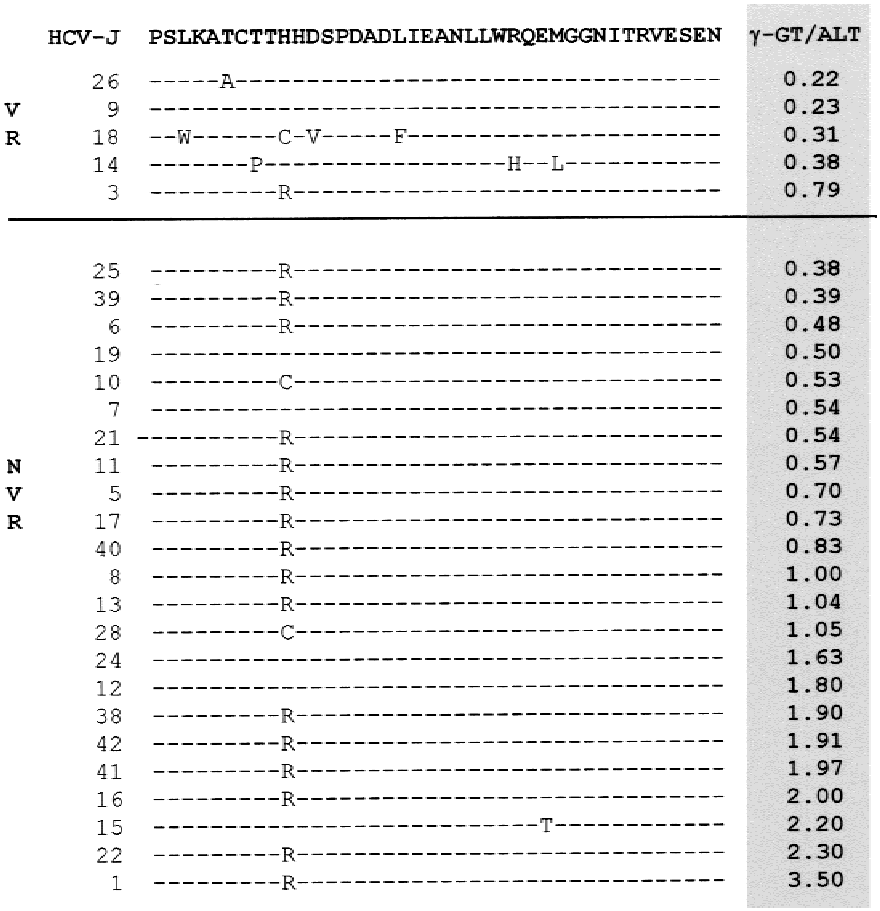

Fig. 2. Evaluation of initial virological response to interferon (IFN)-α therapy in hepatitis C virus (HCV) type 1b-infected patients with respect both to ratio of serum γ-glutamyltransferase/alanine transaminase (γ-GT/ALT) and interferon sensitivity determining region (ISDR) variability. Nucleotide sequences of the ISDR were determined from sera of 28 patients infected by HCV type 1b. Five of the patients were found to respond to IFN-α therapy at least transiently (VR), whereas 23 failed to eliminate viral nucleic acids from serum (NVR). Deduced amino acid sequences were compared with the corresponding prototype sequence of HCV-J. Dotted lines indicate identity with the prototype sequence, amino acid substitutions are given. Serum γ-GT/ALT ratio is indicated. In type 1b-infected patients, virological responsiveness to IFN-α therapy was found to be associated with low serum γ-GT/ALT ratios (<0.4) rather than to differences in ISDR variability.

ity, a prognostic parameter described originally for type 1b-infected patients [Enomoto et al., 1995].

The meaning of the ratio of serum γ-GT to serum ALT activity remains speculative. Under certain circumstances, e.g., ethanol intoxication or cholestasis, the ratio of γ-GT/ALT raises, because γ-GT is an enzyme inducible by ethanol or drugs and solvable by bile acids. However, none of the patients under investigation had cholestasis and patients with actual alcohol abuse had been excluded. Differences in the ratio of serum γ-GT/ALT represent differences in serum ALT activity rather than differences in γ-GT activity (data not shown). ALT is expressed constitutively within the cytoplasm of hepatocytes. Release of ALT into the plasma might result from moderate reversible hepatic lesions, for instance impairment of plasma membrane permeability as it occurs with hydropic swelling of hepatocytes, or from an irreversible destruction of hepatocytes. These two possibilities can be distinguished on the basis of AST activity because AST is associated with the mitochondrial fraction and preferentially released in the condition of complete cellular damage. In HCV infection in general and in the patients under investigation in particular, the low ratio of AST-to-ALT activity indicates mild damage (data not shown). Elevated ALT activity in chronic HCV infection thus reflects mild cellular lesions without substantial destruction.

So far, analyses of ISDR variability with regard to virological responsiveness to IFN-α therapy have been undertaken in patients from Japan, France, Spain, and Germany [Enomoto et al., 1995, 1996; Chayama et al., 1997; Khorsi et al., 1997; Kurosaki et al., 1997; Zeuzem et al., 1997; Sáiz et al., 1998]. ISDR variability is associated with responsiveness to IFN-α and IFN-β in Japanese as well as in Spanish patients infected with HCV type 1b [Enomoto et al., 1995, 1996; Kurosaki et al., 1997; Sáiz et al., 1998]. Recently, Gale et al. [1997] provided experimental evidence for a putative mechanism of ISDR-mediated resistance to IFN-α. These investigators demonstrated that the NS5A protein derived from the IFN-α resistant strain HCV-1a interacts

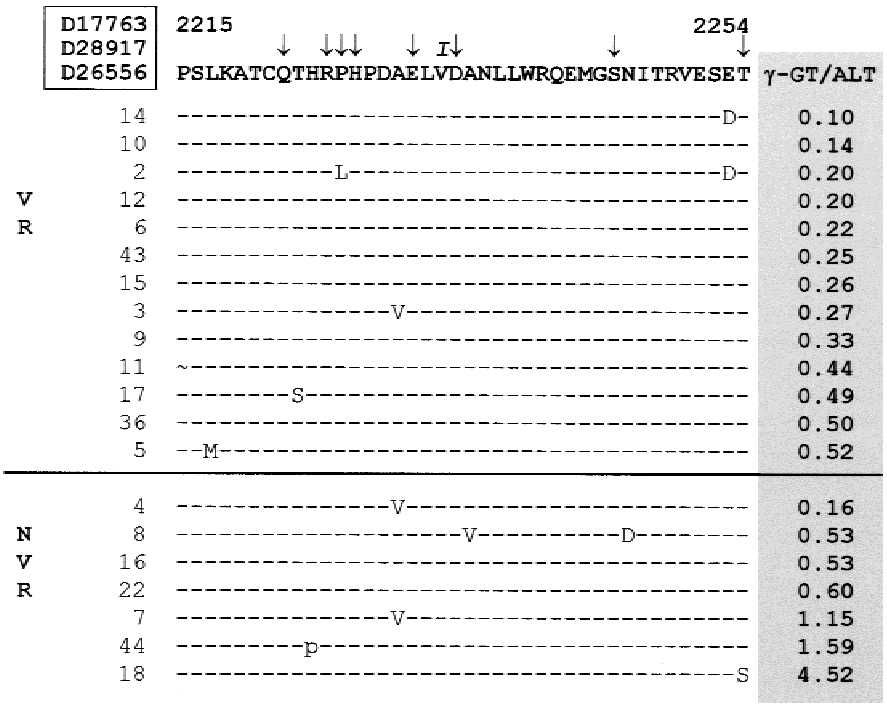

Fig. 3. Analysis of interferon sensitivity determining region (ISDR) variability in hepatitis C virus (HCV) subtype 3a-infected patients and evaluation of its predictive value for virological responsiveness to interferon (IFN)-α therapy. Twenty patients infected with HCV genotype 3a were analyzed with regard to virus ISDR variability. Thirteen of them responded to IFN-α with virus elimination for at least 3 consecutive months during therapy (VR), 7 of them failed to eradicate HCV from the circulation (NVR). Sequences were compared with D28917, D17763, and D26556 database sequences of type 3a isolates. The deduced amino acid sequence of these database sequences differed from HCV-J sequence with respect to 8 or 9 positions as indicated. Dotted lines indicate identity with type 3a database sequence, amino acid substitutions are given. Serum γ-glutamyltransferase/alanine transaminase (γ-GT/ALT) ratio is indicated. It is unambiguously more closely related to IFN-α responsiveness than amino acid variability within the ISDR.

directly with the catalytic domain of the protein kinase PKR, resulting in an inactivation of the enzyme [Gale et al., 1997, 1998]. PKR is an IFN-α-inducible enzyme that is regulated by double-stranded RNA. Its activation is believed to result in the inhibition of replication of a number of viruses, and several viral proteins and RNA species are known to interfere with the activation and catalytic process [reviewed by Jacobs and Langland, 1996]. Thus, the NS5A protein, so far without any known function for HCV replication, might be placed within the group of viral modulators of PKR activity.

However, the results described by the Japanese and the Spanish groups are not concurrent with data from two European groups [Khorsi et al., 1997; Zeuzem et al., 1997]. In French and in German patients, ISDR variability failed to be associated with responsiveness to IFN-α treatment, although identical response criteria were applied. Interestingly, the Japanese and the French groups of patients differed from the German group of patients analyzed by Zeuzem et al. [1997], as well as from our patients in one important aspect, i.e., the proportion of patients with a sustained complete response. Within the Japanese groups, 12/40 (30%) and 31/103 (31%) patients, respectively, fulfilled the criterion of complete sustained response [Chayama et al., 1997; Kurosaki et al., 1997]. This proportion of sustained response was comparable within the French group (17/43, 39.5%). In contrast, only 1/22 (4.5%) patients investigated by Zeuzem et al. [1997] showed a sustained response to therapy. In our own patients, only 2 of 28 (7.1%) infected with HCV type 1b responded to therapy for at least 6 months after IFN-α cessation (data not shown).

In a recent editorial, Herion and Hoofnagle [1997] drew attention to the fact that investigations with a relatively low proportion of sustained complete responding patients do contain a relatively low proportion of patients infected with HCV with high ISDR variability. This observation is also valid for the study presented (Fig. 2). Most of the type 1b-infected patients, 27/28 (96.4%), were found to be infected by viruses with ISDR prototype or intermediate sequences, respectively, and most of these patients failed to respond to therapy. This observation is also valid for two published studies on European [Squadrito et al., 1997] and North American [Hofgärtner et al., 1997] patients.

Regarding type 3a-infected patients, the relative number of responding individuals is clearly higher compared with type 1b-infected patients [Squadrito et al., 1997; Sáiz et al., 1998, and this report]. Kurosaki et al. [1997] compared the rate of complete response to IFN-α of patients infected by type 1b with those infected by types 2a and 2b. Among type 2-infected patients, a rate of response was found that was comparable to type 1b-infected patients with mutant types of ISDR [Kurosaki et al., 1997]. It was pointed out that

prototype sequences of HCV types 2a and 2b differ from prototype genotype 1b in a deletion of 4 amino acids and more than 14 amino acid substitutions within the ISDR [Kurosaki et al., 1997].

Provided that HCV-J prototype virus NS5A protein is indeed an inhibitory modulator of the human PKR enzyme and that amino acid substitutions interfere with its function as experiments by Gale et al. [1997, 1998] might suggest, any amino acid substitution should interfere with activity irrespective of whether substitutions are due to intra- or intergenotype variability.

Data by Zeuzem et al. [1997] on type 1a-infected patients and data presented in this study on type 3a-infected patients (Fig. 3), and those presented by others on type 3a-infected patients [Squadrito et al., 1997; Sáiz et al., 1998] are in accord with this assumption. Zeuzem et al. [1997] analyzed ISDR sequences of 10 type 1a-infected patients. Taking the isolate HCV-1 as a reference for genotype 1a isolates, subtype-specific amino acid sequences differed in no more than 3 positions, reflecting an intermediate type ISDR. Nine of these 10 patients showed 3 or fewer substitutions, and no one responded to the drug. In contrast, genotype 3a database sequences of the putative ISDR differ in 8 and 9 amino acid positions, respectively, from type 1b prototype sequence, and most of the type 3a-infected patients studied were found to respond to treatment (Fig. 3) [Squadrito et al., 1997; Sáiz et al., 1998].

On the basis of these considerations, our data are not necessarily contradictory to the concept that ISDR variability is related to some extent to IFN-α sensitivity. However, the ratio of serum γ-GT/ALT activity in combination with HCV genotype seems to be a more reliable and stringent parameter for discriminating between patients who will show a response to IFN-α therapy in terms of an at least transient elimination of viral nucleic acids from the circulation, and those who will fail to eradicate HCV. Because viral genotypes are determined routinely in many places, the prospectively proved prognostic parameter of serum γ-GT/ALT ratio in combination with the genotype can be taken easily from patients' files without any additional test.

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